

Antiproliferative effects of dibenzocyclooctadiene lignans isolated from *Schisandra chinensis* in human cancer cells

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Abstract—Dibenzocyclooctadiene lignans isolated from *Schisandra chinensis* showed antiproliferative effects in various human cancer cells. The methoxy groups at C-3, C-4, C-3', and C-4', the hydroxyl group at C-8', and the stereo-configuration of the biphenyl ring and the angeloyl group might have influence on these activities. Additional studies indicate that one of mechanism of action of an active compound schisantherin C in A549 human lung cancer cells was related to the inhibition of cell cycle progression in G0/G1 phase.

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Schisandra chinensis (Turcz.) Baill. (Schisandraceae) is widely distributed in northeast Asia (Korea, China, and Japan) and eastern parts of Russia.¹ The fruit of *S. chinensis* was traditionally used in the alleviation or treatment of diseases due to deficiency of lung, heart and kidney, imbalance of Yin and Yang, and impairment of Qi, such as chronic cough, asthma, spontaneous sweating, palpitation, spermatorrhea, diabetes, insomnia, and forgetfulness.^{1,2} Phytochemical studies for the isolation of constituents of *S. chinensis* have been extensively performed since 1970s. Various reports suggested that major bioactive constituents of *S. chinensis* were lignans belong to the dibenzocyclooctadiene type (Fig. 1).³

It has been reported that dibenzocyclooctadiene lignans possess hepatoprotective, antiviral, antioxidant, cytotoxic, and cancer chemopreventive activities.^{1,4–10} The aqueous extract of *S. chinensis* restored hepatic drug

metabolism in a CCl₄-induced hepatotoxicity model.⁴ Gomisin B, gomisin G, and (+)-gomisin K₃ suppressed the formation of surface antigen or e antigen of human type B hepatitis virus.⁵ Gomisin G and other related dibenzocyclooctadiene lignans showed anti-HIV activities.⁶ The structure–activity relationships on the antioxidant and platelet-activating factor (PAF) antagonistic potential were also reported.^{7,8} In particular, together with the range of non-cytotoxic concentration, gomisin A, schisandrin A and B, and schisantherin A restored cytotoxic activities of anticancer agents in multidrug-resistant human cancer cells, and schisandrin B selectively enhanced

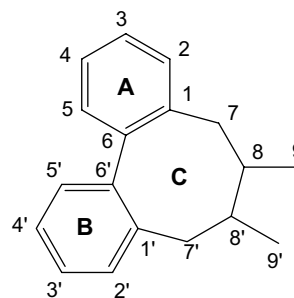


Figure 1. Chemical structures of C₁₈-dibenzocyclooctadiene.

Keywords: Dibenzocyclooctadiene lignan; Schisantherin C; Antiproliferation; Cancer; Cell cycle arrest; A549.

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cytotoxic and proapoptotic effects of doxorubicin.^{9–11} In addition, schisantherin G and propinquinan E were cytotoxic in human cancer cells,¹² and gomisin A inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced tumor formation in a two-stage mouse carcinogenesis model.¹³ These findings imply anticancer and cancer chemopreventive potential of dibenzocyclooctadiene lignans. However, previous studies focused on only one or some lignans. Although schizandrin B induced caspase-3-dependent apoptosis in human hepatoma cells,¹⁴ the mechanisms underlying antiproliferative effects of dibenzocyclooctadiene lignans in human cancer cells have not been studied well. In this study, with the dibenzocyclooctadiene lignans isolated from the fruits of *S. chinensis*, we made an attempt to find the structural requirement for the antiproliferative effect in a panel of several human cancer cell lines (Fig. 2).¹⁵ The mechanism of action of an active compound was also investigated.

Primarily, the inhibitory effects of dibenzocyclooctadiene lignans on the proliferation were evaluated in cultured various human cancer cells (MDA-MB-231, breast cancer (ER⁻); T47D, breast cancer (ER⁺); SK-HEP-1, hepatoma; SNU-638, stomach cancer; HCT-15, colon cancer; K562, leukemia; A549, lung cancer). Cells were treated with various concentrations of dibenzocyclooctadiene lignans for 72 h, and their antiproliferative effects were evaluated using MTT (K562) or SRB (other cell lines) assay.¹⁶ Some dibenzocyclooctadiene lignans from *S. chinensis* showed the growth inhibitory effects against several cancer cells (Table 1). Especially, schizandrin, schisantherin C, and gomisin N showed the effective antiproliferative activities in most of tested cancer cell lines with the IC₅₀ values ranging 10–70 μM. In addition, active compounds exhibited relatively more potent inhibitory effects toward T47D (ER⁺) cells compared with those of MDA-MB-231 (ER⁻) cells. From comparison of their structures with activities, the structural necessity for the antiproliferative effect is suggested as follows. First, the substitution groups of A and B rings might have influence on the antiproliferative activity of dibenzocyclooctadiene lignans in cancer cells. The compounds with the methylenedioxy group at C-3 and C-4 (A ring) or C-3' and C-4' (B ring) tend to show less potent activities than those with methoxy groups at the same positions in comparison of gomisin A and schizandrin, wuweizisu B and schizandrin A, and wuweizisu C and gomisin N, respectively. In addition, the introduction of hydroxyl group at C-8' seems to be influential and generally enhances the activity exemplified with gomisin A and wuweizisu B, and schizandrin and schizandrin A, respectively. Second, the stereo-configuration of rings or side groups might be also an important factor for determining the antiproliferative potential. Compared with gomisin B, schisantherin C, and gomisin C, the stereo-configuration of the hydroxyl group at C-8' and the angeloyl group at C-7' plays an important role in the antiproliferative effect. In case of possessing same side groups, the compound with *R*-biphenyl configuration (for example, wuweizisu B) is less potent than one with *S*-biphenyl configuration (gomisin N).

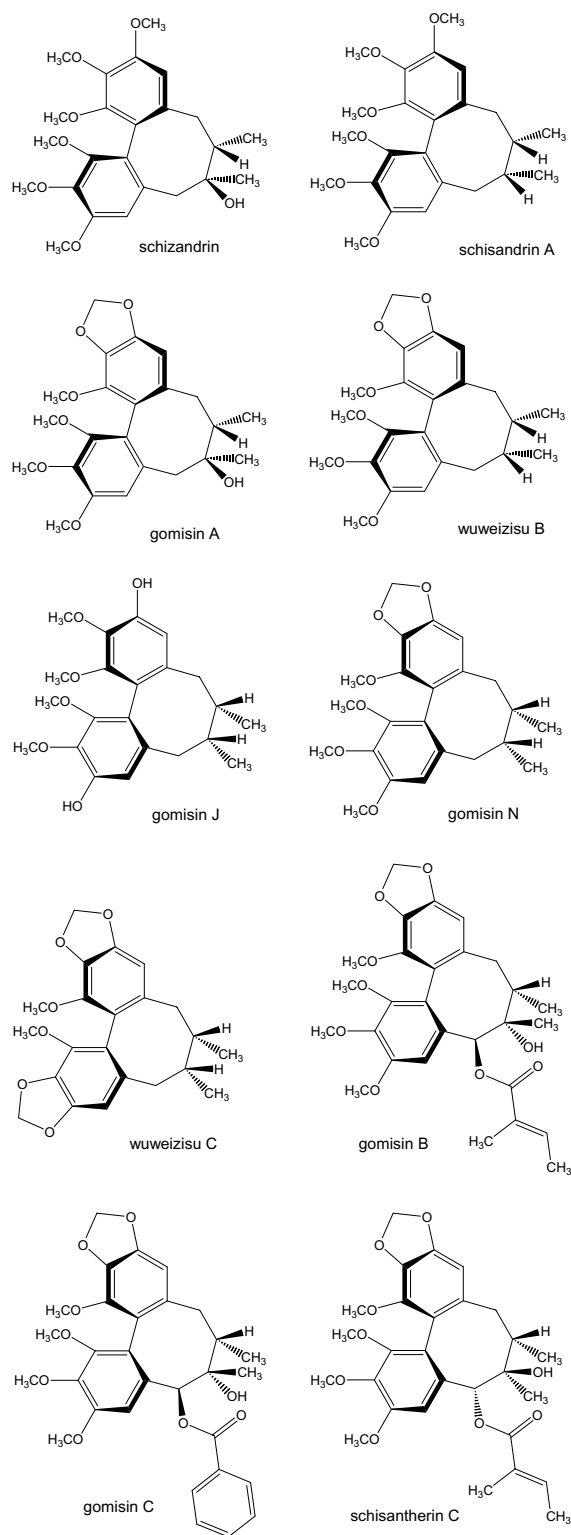


Figure 2. Chemical structures of dibenzocyclooctadiene lignans from *S. chinensis*.

As shown in Table 1, among the tested compounds, schisantherin C showed the most potent inhibitory effect in all of tested human cancer cells. Since schisantherin C was equally effective in A549 and HCT-15 cells, we further investigated the antiproliferative mechanism of

Table 1. Effects of dibenzocyclooctadiene lignans from *S. chinensis* on cell proliferation in human cancer cells

Compound	IC ₅₀ (μM)						
	MDA-MB-231	SK-HEP-1	SNU-638	T47D	HCT-15	K562	A549
Gomisin J	>100	>100	>100	>100	>100	>100	>100
Gomisin N	>100	>100	23.1	19.2	14.1	22.0	21.1
Gomisin A	>100	>100	90.5	>100	>100	>100	84.1
Gomisin B	>100	>100	>100	>100	>100	>100	>100
Schizandrin	61.5	71.3	21.1	19.8	50.2	25.0	20.7
Wuweizisu C	>100	>100	>100	>100	>100	>100	>100
Wuweizisu B	>100	>100	>100	>100	88.1	62.6	87.8
Schisantherin C	33.5	19.9	15.3	26.1	5.6	11.3	5.6
Schisantherin A	>100	42.0	53.1	40.0	>100	>100	>100
Gomisin C	56.4	70.4	44.7	51.2	>100	>100	>100
Doxorubicin	0.2	0.1	0.07	0.1	0.6	NT	0.1

Results are represented as IC₅₀ values (μM). Doxorubicin was used as a positive control. (NT, not tested).

schisantherin C as one representative of dibenzocyclooctadiene lignans in A549 cells.

Inhibition of cell cycle progression and/or induction of apoptosis have been regarded as promising strategies for the control of the proliferation of cancer cells.^{17,18} Therefore, we examined the antiproliferative mechanism of schisantherin C in relation to the regulation of cell cycle progression. A549 cells were treated with various concentrations of schisantherin C (3.75–60 μM) for 24 h, and changes of cell cycle distribution were analyzed by flow cytometry.¹⁶ As depicted in Figure 3, schisantherin C markedly arrested cell cycle progression in a concentration-dependent manner. At 60 μM, more than 70% of total cell population was accumulated in G0/G1 phase, and the cell population in S phase was also gradually decreased. However, the distribution of cells in sub-G1 phase, an indication of cell death, was not increased even at the highest concentration of schisantherin C. Therefore, although the proapoptotic effect of a dibenzocyclooctadiene lignan was reported previously,¹⁴ the mechanism underlying inhibition of cell proliferation by schisantherin C appears to be the arrest of cell cycle progression without cell death in A549 cells.

According to the pronounced inhibitory activity of schisantherin C, the effect of schisantherin C on the expression of proteins related to cell cycle progression was also examined.¹⁹ Treatment of cells with schisantherin C did not affect the expression of cyclin D1, cdk2, and cdk4 (Fig. 4). However, the expression of cyclin E and cyclin A, proteins required for cell cycle transition from G0/G1 phase to S phase and cell cycle progression in S phase, respectively, was decreased by treatment with 60 μM schisantherin C. In particular, schisantherin C induced p27 expression and inhibited phosphorylation of the retinoblastoma protein (pRB) in a dose-dependent manner. It is known that p27 suppresses the kinase activity of cyclin E-cdk2 complex, resulting in blocking phosphorylation of pRB and inhibiting cell cycle progression toward S phase.²⁰ Thus, the down-regulation of cyclin E and up-regulation of p27 lead to the suppression of pRB phosphorylation, and, eventually, contribute to cell cy-

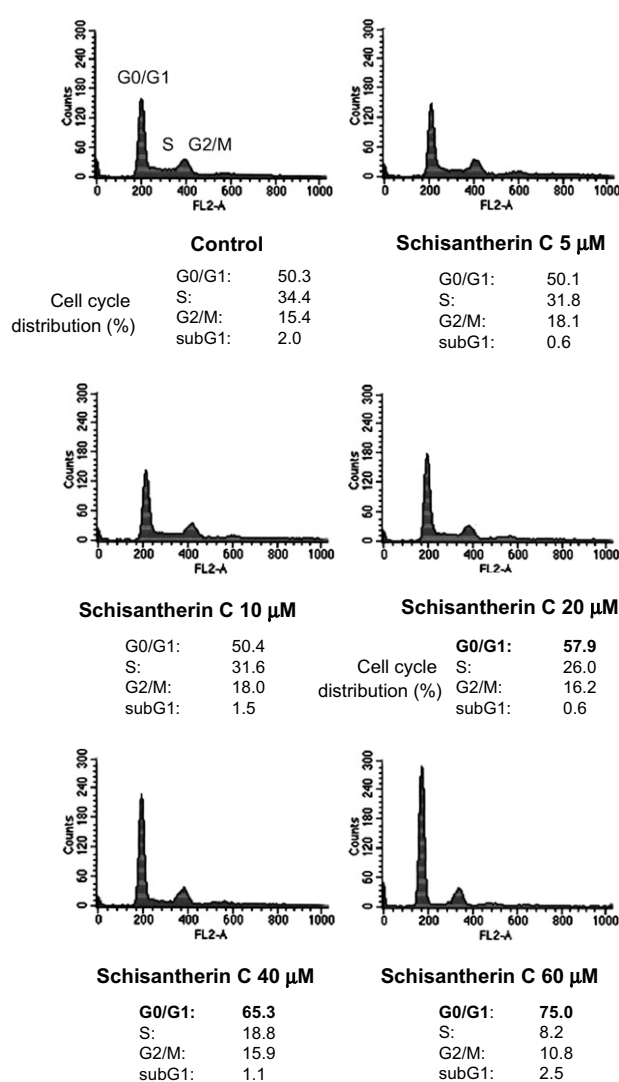


Figure 3. Inhibition of cell cycle progression in the G0/G1 phase of the cell cycle by schisantherin C in A549 cells. A549 cells were treated with various concentrations of schisantherin C for 24 h. Changes of the distribution of cell cycle were analyzed by flow cytometry, and the percentage of distribution in each phase of the cell cycle was determined using ModFIT LT v.2.0 software.

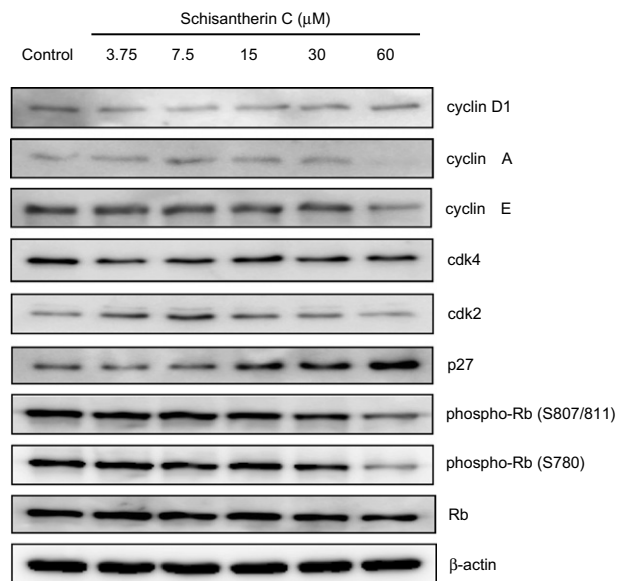


Figure 4. Effects of schisantherin C on the expression of proteins related to cell cycle progression in A549 human lung cancer cells. Cells were exposed to various concentrations of schisantherin C for 24 h. The expression level was evaluated by Western blot analysis.

cle arrest in G0/G1 phase by schisantherin C in A549 cells.

In summary, the present study demonstrates that dibenzocyclooctadiene lignans isolated from the fruit of *S. chinensis* inhibited cell proliferation in human cancer cells, and the antiproliferative mechanism of an active compound schisantherin C appears to be the induction of cell cycle arrest in G0/G1 phase through down-regulation of cyclin E and up-regulation of p27 in A549 human lung cancer cells. These results present the additional biological activity and mechanism of dibenzocyclooctadiene lignans, and also contribute to the development of anticancer agents derived from natural products.

Acknowledgments

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